Are older parents less flexible? Testing age-dependent plasticity in *Nicrophorus vespilloides* burying beetles

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ABSTRACT

Phenotypic plasticity is an important mechanism facilitating adaptation to environmental change that often varies among individuals. One reason for this individual variation is that plasticity may depend on state variables, such as size, condition or age, which affects the costs and benefits of plasticity. Recent theoretical work predicts that plasticity will decrease as an organism ages because costs of plasticity mean that flexible phenotypic adjustments by individuals to environmental change will be less beneficial as age-related survival prospects decrease. Here we use *Nicrophorus vespilloides* burying beetles to test this prediction in the context of parental care. Burying beetles use the carcasses of small vertebrates as resources for breeding and have complex, extended, flexible parental care. Our experiment manipulates female age and (the order of presentation of) carcass size in a repeated-measures design to test the prediction that older beetles are less plastic than younger beetles in parental care. We find evidence in support of our central prediction: young females show greater mean levels of plasticity than older beetles for all traits (parental care, number of offspring, brood mass) except mean larval mass (i.e. size of offspring), with the response to changes in carcass size dependent on the order that young females are presented the carcasses, whereas the response of older females is independent of order. Between-trait correlation analysis indicates that there are age-related trade-offs between the size and number of offspring for older, but not young, mothers. The three age-dependent traits, which are inter-correlated, are also repeatable, indicating potential for co-evolutionary responses to selection.

41 Keywords: Age-dependent, Behavioural plasticity, Life-history, Parental care,
42 Personality, Phenotypic plasticity, Repeatability, State-dependent.
43

INTRODUCTION

Phenotypic plasticity occurs when a genotype expresses different phenotypes in different environments (Pigliucci 2001), and is a potentially important mechanism facilitating organismal adaptation to environmental change (Gomez-Mestre & Jovani 2013). Plasticity is adaptive if it increases the fitness of individuals (Nussey et al. 2007) and there is often considerable variation across individuals in the level of plasticity expressed in response to environmental change (Nussey et al. 2007; Dingemanse et al. 2010; Dingemanse & Wolf 2013; Westneat et al. 2011; Royle et al. 2014). One explanation for this variation in environmental responsiveness is that plasticity is state-dependent (Houston & McNamara 1992; Snell-Rood 2013; Araya-Ajoy & Dingemanse 2017; Moschilla et al. 2018). In particular, state variables, such as size or condition, can affect the costs and benefits of behaviours and therefore how individuals are expected to adjust to changes in current environmental conditions (Snell-Rood 2013; Moschilla et al. 2018), but this idea is rarely tested explicitly.

Empirical work indicates that age may also provide an example of such state-dependent plasticity (Araya-Ajoy & Dingemanse 2017; Abe et al. 2018; Moschilla et al. 2018). A recent model by Fischer et al. (2014) predicts that plasticity will be age-dependent because the costs and benefits of reproduction vary with age: when young and reproductive potential is high then plasticity is favoured, but plasticity should decrease over an individual's lifetime as the potential benefits decrease and costs increase. This is because it does not pay for an individual to adjust their phenotype in response to environmental change unless they can gain the benefits of doing so before they die (Fischer et al. 2014). Parental care traits

have been shown to be plastic in a range of organisms (Westneat et al. 2011; Royle et al. 2014); there is also substantial evidence for costs of reproduction in species with parental care (Alonso-Alvarez & Velando 2012) and for age-related changes in parental care (Creighton et al. 2009; Benowitz et al. 2013; Gomez & Kölliker 2013). In the current study we test for age-dependent plasticity by examining the interplay among individual age, prior reproductive experience and the quantity of breeding resources on parental care behaviours and outcomes in burying beetles.

Nicrophorus vespilloides burying beetles have complex, extended parental care, including active provisioning of offspring, and flexible mating systems with facultative biparental care or uniparental care (either sex) of young (Scott 1998; Royle & Hopwood 2017). Adult burying beetles use the carcasses of small vertebrates, such as mice, as a resource to provision offspring, the availability and quality of which is highly stochastic in the wild (Scott 1998; Royle & Hopwood 2017). The number and size of offspring that they produce is strongly dependent on the size of the carcass that they breed on (Bartlett 1987; Trumbo & Fernandez 1995; Creighton 2005; Creighton et al. 2009). In addition, when individuals breed more than once on carcasses of different sizes the order of presentation of the carcasses affects reproductive output with females breeding on small then large carcasses putting greater investment into their second brood than females breeding in different order combinations (large then small, small then small or large then large; Billman et al. 2014). This is because in species such as burying beetles, where breeding is unpredictable and opportunistic, the passage of time may not always accurately predict the extent of prior investment

94 in reproduction and therefore the ability to invest in future reproduction (as a
95 result of a decline in state), so prior investment per se, not just age, may be an
96 important predictor of current reproductive investment (Billman et al. 2014).
97 However, levels of parental care have also been found to vary with age, with
98 studies generally providing evidence for terminal investment (i.e., an increase in
99 relative investment into current reproduction) with increasing age for mothers
100 (Creighton et al. 2009; Cotter et al. 2011) and fathers (Benowitz et al. 2013),
101 although not always (see Ivimey-Cook & Moorad 2018). Consequently, there is
102 evidence for changes in parental care in response to current environmental
103 conditions (carcass size; the size of breeding resources available to rear
104 offspring) as well as prior environmental conditions (carcass size in the previous
105 breeding attempt), and in response to variation in age. Furthermore, we know
106 that there is individual variation in plasticity of parental care in *Nicrophorus*
107 *vespilloides* (Royle et al. 2014) and other behaviours involved in reproduction
108 (Carter et al. 2015), and that there can be genetic variation underlying this
109 plasticity (GxE; Carter et al. 2015) thus enabling an evolutionary response to
110 selection (Nussey et al. 2007).
111
112 In our experiment we presented carcasses of different sizes to *Nicrophorus*
113 *vespilloides* burying beetles of different ages to test the prediction that plasticity
114 (i.e., the absolute change in phenotypic trait expression) in response to a change
115 in environment (here carcass size) would be greater, on average, in young
116 beetles than older beetles because it is beneficial to be responsive to such
117 variation when residual reproductive value is higher (Fischer et al. 2014).
118 However, investment into current reproduction (parental effort) more generally

is expected to increase with age as the potential for future reproductive prospects decline (i.e., there will be an increase in terminal investment; Williams 1966; Creighton et al. 2009). If there is an effect of prior reproductive investment on current investment we predicted that the order of presentation of carcass size would affect the outcome with females breeding on small carcasses first time around exercising reproductive restraint such that they have greater investment in their second breeding attempt compared to females breeding initially on large carcasses (Billman et al. 2014). Moreover, because previous studies have shown independent effects of age and prior experience on reproductive investment (Creighton et al. 2009; Cotter et al. 2011; Billman et al. 2014), we specifically expected that the order of presentation of carcass size would modify the plastic response in parental effort and reproductive output of young beetles to a greater extent relative to older beetles: we predicted that young beetles would show relatively lower parental effort and produce fewer larvae on small carcasses and have relatively higher effort/output on large carcasses when they first bred on a small carcass compared to when they bred on a large carcass (if there is reproductive restraint associated with costs of reproduction, Williams 1966; Figure 1).

FIGURE 1 HERE

We also estimated the repeatability and among- and within-individual correlations of traits, by pooling repeated measures of individuals (separately for each age class) to partition among- and within-individual (co)variances. Among-individual variation (V_I) can include additive genetic effects (V_A), thus

144 repeatability (V_I/V_P) estimates represent the upper limit to heritability (V_A/V_P)
145 under certain circumstances (Falconer & Mackay 1996; Dohm 2002).
146 Repeatability estimates can therefore provide tentative insights as to the
147 potential evolvability of the traits under test (Dochtermann et al. 2015) and
148 enabled us to quantify consistency of individual performance across different
149 environments (carcass sizes) in relation to the performance of other individuals
150 (Araya-Ajoy & Dingemanse 2017; Houslay et al. 2018). Among-individual
151 correlations represent the genetic and permanent environment effects that are
152 responsible for the associations between traits; within-individual correlations
153 show combined, reversible changes in traits occurring within an individual
154 (Careau et al., 2014; although within-individual correlations may also contain
155 correlated measurement error, Brommer, 2013). We might therefore expect
156 among-individual correlations among all traits, indicating some underlying axis
157 of individual quality in parental care and reproductive output, and for this
158 structure to be conserved across young and old individuals. Within-individual
159 correlations might instead be expected to vary across age classes, reflecting
160 differences among old and young individuals in their phenotypic flexibility
161 across repeated observations.
162
163 **MATERIALS AND METHODS**
164 **General methodology and maintenance**
165 Breeding and maintenance of beetles followed previously established protocols
166 (e.g., Head et al. 2012, 2014; Carter et al. 2015). An outbred stock population of
167 *N. vespilloides* derived from 50 male and 50 female wild beetles caught in
168 Devichoys Wood, Cornwall, UK ($N50^0 11'47" E5^0 7'23"$) during August 2015 was

kept in incubators at 21^{0}C (±1^{0}C) on a 16L:8D hour cycle. Breeding involved random pairing of males and females from different families in individual breeding boxes (17x12x6cm) filled with damp soil and provided with a freshly thawed mouse carcass (15-25g; Livefoods Direct, Sheffield). Once larvae dispersed from the carcass (approximately 1 week after hatching) they were transferred into individual rearing containers (7x7x4cm), given a unique ID and allowed to pupate. Upon eclosion, approximately 3-4 weeks later, beetles were fed two decapitated mealworms (*Tenebrio*) twice weekly until they were sexually mature (ca. 10 days of age). This breeding cycle was then repeated for four generations, with an effective population size of 50 families each generation (Head et al. 2012). Experimental beetles were taken from generation 4. All individuals used were virgin females that were socially naïve before experiments were run. Pronotum width of all beetles was measured three times and averaged, using digital calipers (to 0.1mm), prior to experiments (when 10 days old) in order to quantify size of beetles (Hopwood et al. 2013).

Experimental design

We used a repeated measures cross-over experimental design with individual females of two different age classes breeding consecutively on carcasses of two different sizes (mean±SD): large (24.1±1.46g) and small (12.3±1.12g), manipulating the effect of order within each age class using the cross-over design, where half of the beetles bred first on a small carcass and half on a large carcass (See Figure 1). Our total sample size was N=163 individuals with approximately equal numbers of young and older females. Experimental animals were kept under the same temperature and lighting regime as stock.

194

195 Females were randomly assigned to one of two age classes post-eclosion, which
196 differed by approximately 2 weeks (younger individuals were 10-12 days old at
197 the start of their first breeding attempt and 15-32 days old by the end of their
198 second breeding attempt, while older individuals were 23-25 days old at the
199 start of their first breeding attempt and 37-46 days of age by the end of their
200 second breeding attempt). These age classes are similar to previous work that
201 showed age-dependent effects in parental care (Benowitz et al. 2013) and were
202 chosen to maximise the age difference between treatments in the period before
203 substantial mortality occurs (Hopwood et al*.,* 2013) in order to avoid
204 confounding effects of differential survival *per se* in treatment groups. Prior to
205 breeding, females were mated to randomly selected males from a different
206 family for 48 hours which allowed sufficient time for fertilisation (Müller &
207 Eggert, 1989), before males were removed to avoid potential confounding effects
208 of male parental care, and females were provided with a carcass immediately
209 afterwards. Within each age class individuals were randomly assigned either a
210 large or a small carcass for their first breeding attempt. Following successful
211 breeding individuals were given 48 hours 'rest' before their second breeding
212 attempt (the same male fathering the offspring in both attempts) with a carcass
213 of whichever size class they had not experienced in their first breeding bout. Use
214 of this repeated measures cross-over experimental design allowed us to examine
215 order effects on patterns of investment in relation to age. At larval dispersal,
216 offspring number and brood mass were measured to analyse individual
217 adjustments in brood size to carcass size (Trumbo & Fernandez, 1995; Creighton,
218 2005). Mean larval mass was calculated by dividing brood mass by the number

of larvae in the brood to enable us to assess potential offspring number-size trade-offs (Smiseth et al. 2014; Gasperin & Kilner 2016).

Parental Care observations and measurements of investment

Parental care observations took place around 24, 30 and 40, 48 hours after first larval hatching, when parental provisioning is at its peak intensity. We used instantaneous scan sampling recording individual behaviour every 1 minute for 30 minutes (Head et al. 2012). Before recording observations individuals were acclimatised for a period of 30 minutes (Head et al. 2012). To minimise observer effects on behaviour all observations were recorded under red light conditions using infrared surveillance cameras (N08CX night vision CCTV camera) set up in a 'nicrocosm' (400mm length of black PVC-U Ø 110mm) over the breeding box, with motion detection software (AverMedia NV6240 Express, dvr version 7.7.0.0007; www.avermedia-dvrs.com) to record parental behaviour (for further details see Hopwood *et al*., 2013). Parents were scored as either caring or not caring at each scan; caring can be either direct (regurgitation of carrion to offspring, direct mouth to mouth contact) or indirect (carcass processing and maintenance, moving the carcass and adding antimicrobial secretions)(Scott, 1998; Head et al. 2012), but for the purposes of this study we use a single count of total care provided (=direct+indirect).

At larval dispersal, the number of offspring produced was counted and the whole brood weighed (±0.001g) on an electronic balance (brood mass). These traits provided measures of female performance and patterns of investment in relation to resource availability (carcass size) and age.

244

245 **Statistical analysis**

246 We analysed all data using linear mixed effects models in R version 3.4.1 (R Core
247 Team, 2017). For each model, we used visual inspection to check that we met the
248 assumptions of normality of residuals. We used the 'tidyverse' set of R packages
249 for data handling and visualisation (Wickham et al., 2019).

250

251 *Mean individual plasticity*

252 We used the R package lme4 (Bates et al., 2015) to test for mean individual
253 plasticity in each trait as a function of age, carcass size and order of carcass size
254 type. For each response type in turn (i.e., parental care, brood mass, number of
255 offspring, mean larval mass) we fit a linear mixed effects model that included
256 main effects and all interactions among age, carcass size, and carcass order. The
257 model also included a covariate of beetle size (mean-centred and scaled to
258 standard deviation units), and female ID as a random effect. For mean larval
259 mass only, we also included the number of offspring (mean-centred) as a further
260 covariate. We tested the significance of fixed effects terms by removing a term
261 (starting with the highest-order interaction), and comparing this updated model
262 to the previous one (both fitted with ML) using a likelihood ratio test (LRT). We
263 do not provide p-values for main effects or lower-order terms that are part of
264 significant higher-order interaction terms as these cannot be removed from the
265 model in isolation in lme4.

266

267 *Age-specific among- and within-individual correlations*

We used the R interface package ASreml-R (Gilmour et al., 2009) to estimate the repeatability of each trait and the among- and within-individual (co)variances between our 4 traits of interest (i.e., parental care, total brood mass, number of offspring, and mean larval mass), separately for each age class. For all response traits, observations were mean-centred and standardised to units of 1 standard deviation across the whole data set (allowing comparison of (co)variances across contexts and traits). Each trait was considered to follow a normal error distribution, based on visual checks of univariate models above. In each age-specific model we fit trait-specific effects of carcass size, order of presentation, the interaction between these terms, and beetle body size. We fit unstructured covariance matrices for the random effect of individual ID (thus estimating variances and covariances at the among-individual level) and for the residuals term (within-individual level). We used a parametric bootstrapping procedure to estimate 95% confidence intervals around each (co)variance term (e.g., Houslay et al., 2018). We scaled covariances to correlations for presentation purposes using the standard form (i.e., $r_{1,2} = COV_{1,2} / \sqrt{V_1.V_2}$). We consider correlations where these confidence intervals exclude zero to be nominally significant, although we caution that the estimated intervals are necessarily approximate and based on the assumption of multivariate normality (see Houle & Meyer, 2015). We calculated the standard errors of (co)variances and correlations using the 'pin' function provided in the R package nadiv (Wolak, 2012).

Ethical note

Our experiments comply with the ASAB guidelines for the use of animals https://www.asab.org/ethics and were approved by the University of Exeter,

College of Life and Environmental Sciences, Penryn, Ethics Committee (application number 2016/1200). Our experiments addressed the 3Rs through the use of an invertebrate species, rather than a species protected under ASPA. Furthermore, we minimised potential stress in our experiments by reducing sample sizes whilst maintaining sufficient statistical power to detect effects of interest and by refining our experimental design; for example, by monitoring behaviours remotely using motion-detection cameras.

RESULTS

Mean individual plasticity

The amount of time spent providing parental care is dependent on effects of age, carcass size and the order of carcass presentation (age x carcass x order interaction: $\chi^2_1 = 6.4$, $P = 0.011$; Table 1A). While older females show similar patterns across the order groups (providing slightly higher care to broods at small relative to larger carcasses), younger females show lower levels of care on larger carcasses relative to small carcasses when large is presented first (Figure 2A). Younger females also show lower levels of care relative to older females on whichever carcass is presented first. Female body size is not related to parental care provision ($\chi^2_1 = 0.59$, $P = 0.44$).

TABLE 1 HERE

For total brood mass, we find that older females again show similar patterns across the order groups, producing broods of greater mass on larger carcasses relative to small ones (age x carcass x order interaction: $\chi^2_1 = 16.4$, $P < 0.001$;

318 Figure 2B; Table 1B). Young females produce broods of similar total mass on
319 each carcass size when presented with the large carcass first and small carcass
320 second; however, when presented with the small carcass first they produce a
321 relatively small brood mass, and a much larger brood mass on the second, large
322 carcass. The total brood mass produced by large females is greater than that of
323 smaller females (body size: $\chi^2_1 = 12.7$, $P < 0.001$).
324
325 We also find a significant three-way interaction among age, carcass and carcass
326 order on the number of offspring produced ($\chi^2_1 = 21.9$, $P < 0.001$; Figure 2C;
327 Table 1C). When presented with the large carcass first, both young and older
328 females produce slightly more offspring on larger carcasses. Meanwhile, when
329 smaller carcasses are presented first, young females reduce the number of
330 offspring on small carcasses, and increase the number on large carcasses. Larger
331 females produce a greater number of offspring than small females (body size: χ^2_1
332 $= 7.93$, $P = 0.005$).
333
334 We do not find a significant three-way interaction effect on the mean larval mass
335 (age x carcass x order interaction: $\chi^2_1 = 1.6$, $P = 0.203$; Figure 2D; Table 1D,
336 although there are significant interactions between carcass size and age ($\chi^2_1 =$
337 45.3, $P < 0.001$), and carcass size and order ($\chi^2_1 = 22.1$, $P < 0.001$). Older females
338 tend to produce offspring of greater average mass on large carcasses
339 (irrespective of carcass order), whilst mean offspring mass is largely
340 independent of carcass size in young females. Mean offspring mass is the same
341 across both carcass sizes when parents rear offspring on large carcasses first, but
342 is greater on large carcasses when parents rear offspring on small carcasses first.

343 Larger females have slightly larger larvae than small females, although this is
344 marginally nonsignificant (body size: $\chi^2_1 = 3.82$, $P = 0.051$), and there is no effect
345 of the number of offspring on mean larval mass ($\chi^2_1 = 0.25$, $P = 0.62$).
346
347 FIGURE 2 HERE
348 *Age-specific among- and within-individual correlations*
349 We find strong and significant positive correlations between number of offspring
350 and total brood mass, both among and within individuals (Table 2). There are
351 strong positive among-individual correlations between the amount of time spent
352 providing parental care and both the number of offspring and brood mass for
353 young but not older beetles. Within-individual correlations between parental
354 care and offspring number are significantly, but weakly, positive for both young
355 and older beetles but the within-individual correlation between parental care
356 and brood mass is only significantly positive for the older, not young, beetles.
357 Furthermore there is a strong negative among-individual correlation between
358 the number of offspring produced and mean larval mass for older females, but no
359 correlation in young females (Table 2; although we note that the confidence
360 intervals for among-individual variation in mean larval mass do slightly overlap
361 zero, so caution should be applied to this result). In contrast, there is no
362 significant within-individual correlation between the number of offspring and
363 mean larval mass for beetles in either age class. Brood mass was positively
364 correlated with the number of offspring produced and the mean larval mass
365 within individuals, for both older and young females.
366
367 TABLE 2 HERE

Adjusted repeatabilities (i.e., the proportion of phenotypic variation explained by consistent individual differences after accounting for fixed effects) were significant for all reproductive traits except mean larval mass (estimates given with bootstrapped 95% confidence intervals; parental care: old = 0.23 (0.02, 0.45), young = 0.27 (0.07, 0.46); number of offspring: old = 0.56 (0.30, 0.79), young = 0.33 (0.16, 0.50); brood mass: old = 0.28 (0.09, 0.46), young = 0.25 (0.07, 0.43); mean larval mass: old = 0.11 (-0.04, 0.25), young = 0.18 (-0.03, 0.42)).

DISCUSSION

Our results provide empirical support for age-dependent plasticity in parental care and reproductive output in *Nicrophorus vespilloides*. As predicted by theory (Fischer et al. 2014), young females show greater mean levels of plasticity (i.e., absolute difference in phenotypic trait expression in response to carcass size variation) than older females for all traits except mean larval mass. In particular, the mean plastic response to carcass size indicates that in young, but not older, females it is contingent on the order of presentation, with evidence for reproductive restraint in young females: the difference between young and older females in parental care, offspring number and total brood mass is greatest when small carcasses are presented first and is minimised when large carcasses are presented first. In contrast, older females are more invariant in their population mean responses to carcass size (i.e., responses are largely independent of order effects).

This order effect provides support for the asset protection principle (Clark 1994), which states that individuals with higher residual reproductive value (i.e., young females) should be less willing to engage in risky behaviours (such as high parental investment into low value resources) because they will pay a greater cost to their future reproductive potential compared to individuals with lower residual reproductive value (older females). Little is known about how many times individual burying beetles breed in the wild. Carcass availability is unpredictable in time and space (Scott 1998; Royle & Hopwood 2017), which might be expected to reduce encounter rates, but calculations indicate that rodent population densities are likely to be sufficiently robust during the

401 breeding season that a mortality rate of as little as 1-2% would be more than
402 enough to sustain beetle populations (Smith & Merrick 2001) and that's without
403 taking into account the availability of carcasses of small birds, which burying
404 beetles also use. In addition, there is considerable evidence from experiments in
405 the lab that burying beetles breed multiple times, and, in the lab and the wild,
406 behave as if they have a future (i.e., adjust patterns of investment to cues such as
407 carcass size, age and social interactions that provide information about likely
408 future prospects; Bartlett 1987; Benowitz et al. 2013; Creighton et al. 2009;
409 Billman et al. 2014, Hopwood et al. 2016). Consequently there is ample scope for
410 the evolution of age-dependent plasticity in burying beetle reproduction, which
411 is supported by our results.
412
413 Similar age-dependent plasticity has also been found in Australian field crickets
414 (*Teleogryllus oceanicus*) in the context of risk-taking behaviours: younger
415 females are more risk averse (Moschilla et al. 2018). As also expected if there is
416 terminal investment (Creighton et al. 2009), older females in our study generally
417 spent more time providing parental care compared to younger females,
418 mirroring previous work on male *Nicrophorus vespilloides* (Benowitz et al. 2013).
419 Correlational analyses also reveal that the relationship between amount of time
420 spent providing parental care and reproductive output is less consistent in older
421 than in young beetles. Among-individual correlations show strong positive
422 relationships between parental care and number of offspring and parental care
423 and brood mass respectively, but only for young mothers. In contrast, within-
424 individual correlations for the same combinations of traits are slightly stronger
425 (if broadly similar) for older mothers compared to young mothers. However,

426 despite this age-dependent variation in patterns of parental care there is no
427 overall difference in reproductive performance (brood mass, number of
428 offspring, mean larval mass) between the age classes. Together these results
429 indicate that parental care provision is just as effective (in terms of reproductive
430 output), but is more variable, and possibly more costly, for older beetles than
431 young beetles.
432
433 It is unclear whether these age-dependent patterns are due to the effects of
434 senescence *per se* because our 'old' beetles were not especially old (Hopwood et
435 al. 2013; Ivimey-Cook & Moorad 2018), even though they are at an age (~40-
436 45d) when future reproductive prospects are reduced as a result of an imminent
437 decline in survival probability. Intrinsic mortality rates in our lab population are
438 low until ~50 days of age but then accelerate quite rapidly so that 50% of
439 individuals are dead by 100-116 days (Hopwood et al. 2013). This age at death is
440 likely to represent the upper limit to survival as it does not include the effects of
441 extrinsic mortality that would be found in natural populations, so the clock will
442 effectively be ticking loudly for the 'older' beetles in our experiment, even if they
443 are not very old.
444
445 Trait correlations from our study provide further supporting evidence that older
446 beetles respond differently to variation in resource availability compared to
447 young beetles and may be subject to constraints leading to trade-offs. We find a
448 strong negative among-individual correlation between the number of offspring
449 and size of offspring (mean larval mass) for older, but not young, beetles
450 (although we caveat that the level of among-individual variation in mean larval

mass was nominally non-significant for both age classes). Previous studies on *Nicrophorus vespilloides* have generally also found evidence for a negative relationship between number and size of offspring (e.g., Smiseth et al. 2014; De Gasperin & Kilner 2016; although not always, see De Gasperin & Kilner 2016). The current study differs from these previous studies in explicitly examining age-dependent effects while statistically controlling for the effects of other factors known to impact the shape of this relationship, such as carcass size and female size (Smiseth et al. 2014). Our data therefore provide some evidence for the existence of age-dependent trade-offs between the number and size of offspring. Without explicitly testing for differences in costs of parental care between beetles in the two age classes it is difficult to be sure if older females are physiologically constrained (indicating senescence), whether it reflects a difference in reproductive 'strategy', or is a combination of both.

Regardless of whether physiological constraints are involved or not, the data indicate that the age of the mother is associated with variation in – and covariation between – number and size of offspring produced.

This difference in the relationship between the number and size of offspring produced in relation to the age of the mother has implications for parent-offspring conflict. A trade-off between the number and size of offspring can generate parent-offspring conflict because selection favours mothers that produce lots of offspring (even at the expense of offspring size) but simultaneously favours offspring that can achieve a large size due to less competition over resources from fewer siblings (Wilson et al. 2005; De Gasperin & Kilner 2016). Parent-offspring conflict is therefore likely to be higher in broods

476 of older beetle mothers than broods of young mothers. However, while it is
477 reasonably safe to assume that mothers will benefit from producing more
478 offspring it is not necessarily the case that larger offspring will have higher
479 fitness than small offspring. This is because the benefits of size for fitness are
480 context-dependent in *N. vespilloides* in the wild (Hopwood et al. 2016; Royle &
481 Hopwood 2017). Nevertheless, if a population is age-structured this difference in
482 the shape of the offspring number-size relationship between old and young
483 mothers may be important. It has the potential to affect the dynamics of the
484 population through transgenerational alterations in the size profile of
485 individuals, which may affect the socio-ecology (Royle & Hopwood 2017). For
486 example, an increase in older females in the population might lead to more
487 offspring produced for a given size of carcass and lead to higher beetle density
488 and greater competition for carcasses in the next generation, which would then
489 put a premium on large size.
490
491 While we find plastic effects in response to the combination of age, carcass and
492 order size, we also find repeatability for all traits except for one. This indicates
493 that while there are population-level effects in how beetles of different age
494 classes respond to a change in carcass size, individuals also differ consistently in
495 their overall response: those that tend to have high values in one environment
496 also tend to have relatively high values in the other environment. We find
497 evidence of consistency in response to carcass size (repeatability of effort and
498 output) for parental care, brood mass and the number of offspring but not larval
499 mass, with high repeatability for number of offspring in particular. Repeatability
500 for parental care is lower than that for number of offspring and brood mass but

501 is within the range typically seen in studies of animal personality (Bell et al.
502 2009). Given that parental care involves behaviour it can be concluded that
503 female burying beetles differed consistently in their parental care 'personality'
504 and/or individual 'quality', such that individuals that put in a relatively high
505 amount of effort on a small carcass also put in a relatively high amount of effort
506 on a large carcass. This interpretation supports the results of our between-trait
507 correlations and concurs with other studies on consistency of parental care,
508 mainly in birds, which show that parental care is repeatable (Schwagmeyer &
509 Mock 2003; Nakagawa et al. 2007; Westneat et al. 2011; Barbasch & Buston
510 2018) and in the case of *N. vespilloides*, heritable (Walling et al. 2008; Head et al.
511 2012).
512
513 The repeatability of parental care, offspring number and brood mass, in addition
514 to the presence of significant among-individual correlations between traits,
515 indicates the potential for genetic (co)-variation underlying the phenotypic
516 (co)variance we observe. If so, selection on one trait in one environment could
517 lead to correlated responses in another trait. There is known to be heritable
518 variation underlying parental care traits in *N. vespilloides* (Walling et al. 2008;
519 Head et al. 2012) and associated correlated evolution with other traits involved
520 in reproduction (Head et al. 2014). Given the differences in among-individual
521 correlations between traits at distinct age classes, a further avenue of research
522 could therefore be to investigate whether any genetic covariation among
523 parenting traits also depends upon age, and indeed how single traits are
524 correlated across age classes at the genetic level (i.e., the existence of genotype x
525 age interactions). The structure of the genetic variance-covariance matrix (**G**) is

not necessarily static as organisms age (Class & Brommer 2015), so any evolutionary response to selection on traits may be determined by how **G** is shaped as a result of both internal as well as external processes.

In conclusion, our predictions were largely supported, providing evidence for age-dependent plasticity in *N. vespilloides*: young beetles are more responsive to changes in environment (carcass size) and the order in which the environments were encountered, for all traits except mean larval mass. Older beetles also spend more time providing care, but this is not accompanied by a corresponding increase in reproductive success (older beetles do not produce more or larger offspring). This, in addition to a significant offspring size-number trade-off in older, but not young, mothers suggests that costs of care increase with age and/or that individual differences in quality or condition (as indicated by the patterns of individual variance and repeatability of parental care and reproductive output) become magnified over time.

Author's contributions

NJR and PAK came up with the idea and designed the experiments. PAK performed the experiments. TMH and PAK analysed the data. NJR led the writing with all authors contributing to interpreting and writing up the results.

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Data accessibility

Data are deposited in the University of Exeter repository (ORE) and freely available at https://doi.org/10.24378/exe.2143.

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716 Table 1. Testing mean individual plasticity due to combined effects of age, carcass size and order of carcass size presentation.

717

	Parental care					Brood mass					Number of offspring					Mean larval mass				
Terms	**Estimate**	**SE**	**t**	**χ^2_1**	**P**	**Estimate**	**SE**	**t**	**χ^2_1**	**P**	**Estimate**	**SE**	**t**	**χ^2_1**	**P**	**Estimate**	**SE**	**t**	**χ^2_1**	**P**
Intercept	41.94	5.34	7.8			4.12	0.21	19.3			24.48	1.27	19.2			0.166	0.004	40.7		
Carcass size (small)	11.61	6.43	1.8			-1.21	0.25	-4.9			-3.66	1.22	-3.0			-0.020	0.005	-3.7		
Age (younger)	-18.72	7.69	-2.4			0.57	0.31	-1.9			0.05	1.83	0.0			-0.024	0.005	-5.1		
Order (small-large)	8.41	7.42	1.1			0.19	0.30	0.6			-0.40	1.77	-0.2			0.016	0.005	3.5		
Beetle size	1.65	2.18	0.8	0.6	0.44	0.32	0.09	3.6	12.7	<0.001	1.60	0.57	2.8	7.9	0.005	0.004	0.002	1.9	3.8	0.051
Carcass size x Age	13.89	9.25	1.5			0.92	0.35	2.6			-1.37	1.75	-0.8			0.047	0.006	7.2	45.3	<0.001
Carcass size x Order	0.72	8.92	0.1			0.01	0.34	0.0			3.16	1.69	1.9			-0.03	0.006	-4.8	22.1	<0.001
Age x Order	7.30	10.80	0.7			1.04	0.43	2.4			5.31	2.58	2.1			N/A				
Carcass size x Age x Order	-32.82	12.90	-2.5	6.4	0.011	-2.04	0.50	-4.1	16.4	<0.001	-11.71	2.45	-4.8	21.9	<0.001	N/A				
Number of offspring	N/A					N/A					N/A					-0.0001	0.0002	-0.5	0.2	0.62

718

719 Parameter values and t statistics for fixed effects are given from mixed models for each trait; parental effort, total brood mass, number
720 of offspring, and mean larval mass. Chi-square test statistics and p-values are given for the highest order interaction(s) remaining in the
721 simplified model (see text for details), and for beetle size (covariate not involved in interactions). Parameter estimates are given from
722 the final model after simplification (non-significant interactions are thus denoted N/A). Age, order and carcass size terms are estimated
723 as deviations from a reference group (older beetles on large carcasses with order large-small). Number of offspring was used as an
724 additional covariate in the model for mean larval mass only.
725
726

727 Table 2. Estimates (and 95% bootstrapped confidence intervals; see text for details) of among-individual variance (diagonals, plain text),
728 within-individual variance (diagonals, italics), between-trait correlations at the among-individual (above diagonal) and within-
729 individual (below diagonal) levels, separately for older and younger individuals.

730

Old	% care	# offspring	Brood mass	Mean larval mass	***Young***	% care	# offspring	Brood mass	Mean larval mass
% care	0.23 (0.01,0.44) *0.73 (0.52,0.95)*	0.09 (-0.45,0.58)	0.09 (-0.69,0.69)	-0.04 (-1.4,1.0)	% care	0.27 (0.07,0.46) *0.58 (0.40,0.77)*	**0.73 (0.35,1.16)**	**0.77 (0.22,1.38)**	0.15 (-0.70,1.30)
# offspring	**0.27 (0.06,0.47)**	0.56 (0.32,0.80) *0.40 (0.28,0.52)*	**0.99 (0.93,1.07)**	**-0.90 (-2.1,-0.3)**	# offspring	0.22 (-0.02,0.45)	0.32 (0.15,0.50) *0.35 (0.24,0.47)*	**0.93 (0.83, 1.02)**	-0.13 (-0.95,0.55)
Brood mass	**0.34 (0.14,0.53)**	**0.91(0.86,0.94)**	0.28 (0.09,0.45) *0.24 (0.36,0.67)*	-0.83 (-2.57, 0.02)	Brood mass	0.12 (-0.12,0.34)	**0.86 (0.79,0.92)**	0.25 (0.07,0.43) *0.49 (0.34, 0.65)*	0.23 (-0.76, 0.87)
Mean larval mass	0.14 (-0.08,0.34)	0.13 (-0.09,0.33)	**0.46 (0.29,0.62)**	0.11 (-0.05,0.25) *0.53 (0.39,0.69)*	Mean larval mass	-0.18 (-0.39,0.06)	0.22 (-0.01,0.45)	**0.64 (0.49,0.77)**	0.18 (-0.04,0.41) *0.78 (0.50, 1.01)*

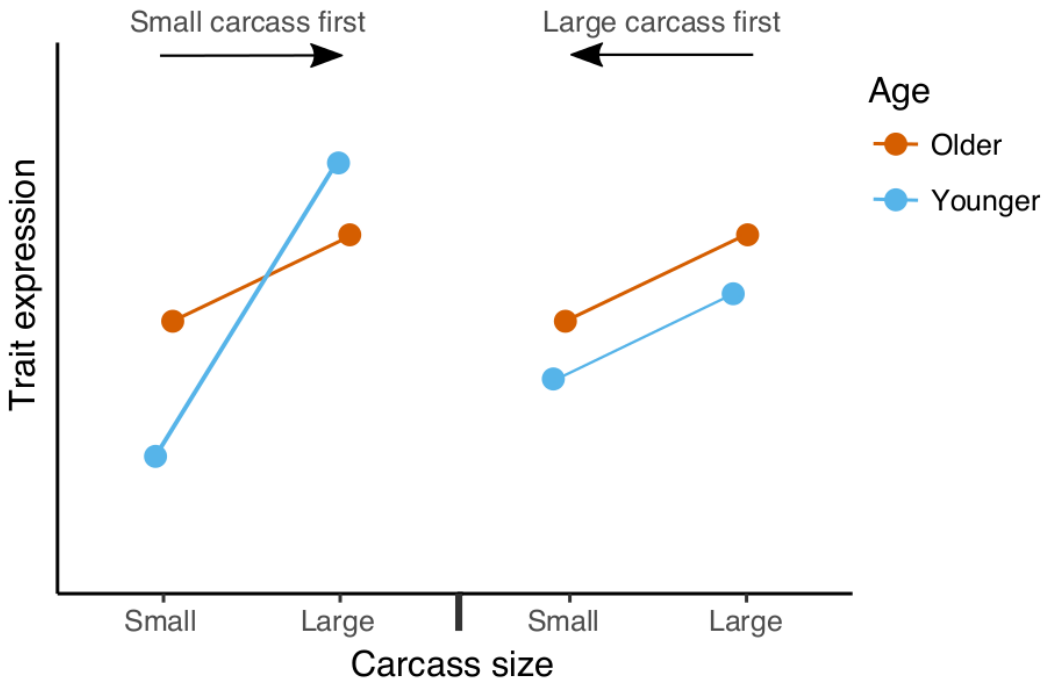
731

732 We estimated all variances and correlations using multivariate mixed models (one for each age class), after accounting for average
733 effects of carcass size, order of presentation, the interaction between these effects, and individual beetle size.

734 **Figures**

735

736 Figure 1. Illustration of trait expression predictions in relation to carcass size,
737 order of presentation of carcasses and age of beetles. Arrows above indicate the
738 order that carcasses were provided for breeding. See main text for further
739 details/rationale for predictions.



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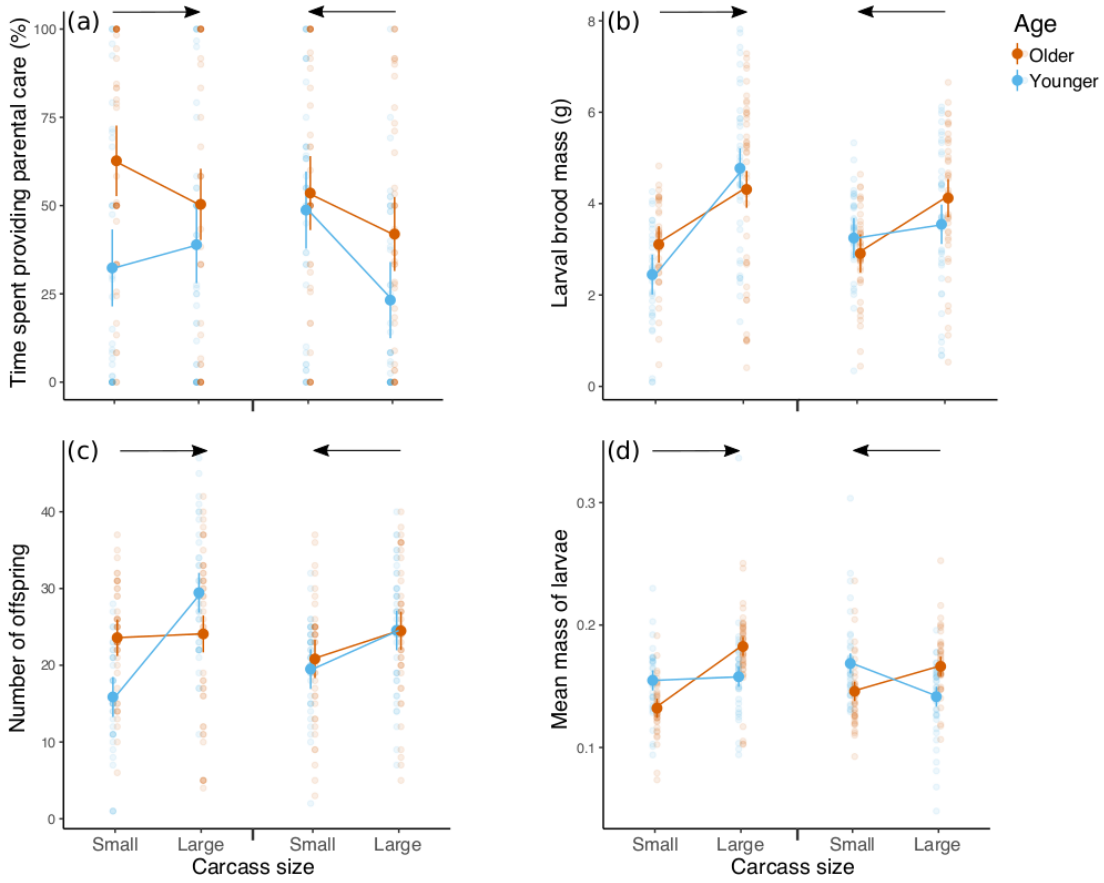
742

743 Figure 2. Panels show the effects of age, carcass size and order of presentation on
744 (a) parental effort, (b) total brood mass, (c) number of offspring, and (d) mean
745 larval mass. Large points and vertical bars give the estimates as predicted from a
746 linear mixed model analysis with their 95% confidence intervals (calculated as
747 1.96 times the standard error, incorporating fixed effects variance only). Faint
748 points show raw data. The arrows above each panel indicate the order of
749 presentation of carcass.



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